PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Wen-Tien CHEN

Serial No.: 09/541,785

Filed: April 3, 2000

For: COMPOSITIONS AND METHODS FOR INHIBITION OF CANCER INVASION AND ANGIOGENESIS

Examiner: Brumback, B.

Group Art Unit: 1642

Attorney's Docket: 178-295

Dated: July 30, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

STATEMENT UNDER 37 C.F.R. §1.804(b)

Sir:

The Rat Hybridomas, E19 and E26, which produce the monoclonal antibody E19 and monoclonal antibody E26, respectively, were deposited with the American Type Culture Collection, Manassas, VA 20110 on May 15, 2001 are the same hybridomas referred to by these names in the present specification as filed on April 3, 2000 and also in the related prior provisional application 60/193,987 filed on April 1, 2000. The above-identified hybridomas and monoclonal antibodies were in Applicants possession prior to filing of each of the above-identified applications.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Wen-Tien Chen, Ph.D.

Date: July 30, 2002

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Group Art Unit: 1643

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Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR 1.132

Sir;

I, Wen-Tien Chen, Ph.D., declare as follows:

1. I received a Ph.D. in Genetics, Cellular and Developmental Biology from Yale University in 1979.

2. Since 1982, I have held faculty positions at a number of accredited universities, including the Howard University Cancer Center at Howard University College of Medicine, the Department of Cell Biology at Georgetown University School of Medicine, and the Lombardi Cancer Center at Georgetown University School of Medicine.

3. Currently, I am a professor in the Department of Medicine/Division of Neoplastic Diseases at the State University of New York at Stony Brook, New York. In addition, I am the director for the Program of Angiogenesis and Metastasis at the Long Island Cancer Center at the State University of New York at Stony Brook.

4. I served as a committee member for the National Institutes of Health in review panels for Biomedical Sciences Study Section from 1984 to1987 and Cell Biology Study Section from 1989 to 1993.

5. For various periods from 1984 to 2002, I served as an ad hoc committee member for the following: Cancer Center Support Grants, site visits; National Cancer Institute Cancer Preclinical Program Project Review Committee, site visits; National Cancer Institute Special Review Committee, Outstanding Investigator Grants; national Institutes of Health Special Review Committee; RFA De-98-008 Genetic Mechanisms in Oral Cancer; RFA 86-HL-30-L Fibroblast Heterogeneity in Pulmonary Fibrosis: and National Pathology B Study Section, Special Study Section Review.

6. I am an author of over twenty peer-reviewed publications concerning cancer invasion and angiogenesis.

7. I am an expert in the field of cancer invasion and angiogenesis as evidenced by my curriculum vitae, which is attached as Exhibit 1.

8. I am the sole inventor of the above-identified U.S. patent application Serial No: 09/541,785 titled "Compositions and Methods for Inhibition of Cancer Invasion and Angiogenesis."

9. Over one hundred monoclonal antibodies directed against human placental dipeptidyl peptidase IV (DPPIV) have been produced in my laboratory under my direct supervision and control.

10. Only twenty-six of the more than one hundred monoclonal antibodies directed against the DPPIV epiptope of the DPPIV-seprase complex were determined by Western Blot analysis to bind to DPPIV with high affinity.

11. I have investigated the ability to inhibit angiogenesis of the twenty-six high affinity monoclonal antibodies directed against the DPPIV-seprase complex described above and three high affinity monoclonal antibodies directed against CD26 available from other sources. These twenty-nine high affinity monoclonal antibodies are listed in Exhibit 2.

12. The twenty-nine monoclonal antibodies shown in Exhibit 2, include the twenty-six high affinity antibodies produced in my laboratory (E1-F102), the 1F7 antibody directed against CD26 and disclosed in Duke-Cohan et al., WO 96/38550 cited by the Examiner, and two commercial preparations also directed against CD26 (202.36 from Ancell and M-A261 from PharMingen).

13. Angiogenesis involves degradation of collagen and cellular invasion. Degradation of gelatin containing denatured collagen is used as one model for angiogenesis. Another model for angiogenesis is invasion of endothelial cells in type 1 collagen and matrix gels.

14. The high affinity monoclonal antibodies listed in Exhibit 2 were tested for their ability to inhibit angiogenesis by examining gelatin degradation and invasion of endothelial cells in type I collagen and matrix gels.

15. Gelatin degradation by the DPPIV-seprase complex was determined by measuring the increase in fluorescence in a mixture of protease complexes and fluorescently labeled gelatin. The experimental protocol for measuring such proteolytic activities of protease complexes is detailed in Exhibit 3. The ability of the high affinity monoclonal antibodies to inhibit

the release of fluorescent-labeled gelatin by immunocaptured DPPIV-seprase complexes was measured. The results are listed in Exhibit 2.

16. Invasion was determined by the inhibition of endothelial cell migration in type I collagen and matrix gels. The experimental protocol for determining invasion of endothelial cells in collagen and matrix gels was described in the legend for Figures 2h-j and Example 4 of the specification of U.S. Serial No. 09/541,785 as originally filed on April 3, 2000.

17. Of the high affinity monoclonal antibodies listed in Exhibit 2, only two monoclonal antibodies (i.e., E19 and E26) exhibited anti-angiogenesis properties as evidenced by inhibition of gelatin degradation and inhibition of endothelial cell migration in type 1 collagen and matrix gels.

18. Figures 2h-j of U.S. Serial No. 09/541,785 as originally filed on April 3, 2000 demonstrate that the high affinity monoclonal antibody E3 did not inhibit endothelial cell migration. In contrast, Figures 2h-j show that the high affinity monoclonal antibodies E19 or E26 inhibited endothelial cell migration.

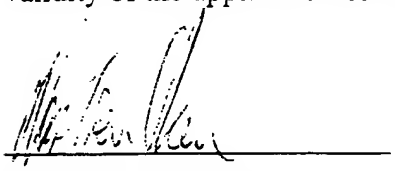
19. Thus, only E19 and E26 of the twenty-nine high affinity monoclonal antibodies from over one hundred monoclonal antibodies inhibited

angiogenesis. Accordingly, the ability of the E19 and E26 monoclonal antibodies to inhibit angiogenesis is a rare property among monoclonal antibodies directed against DPPIV.

20. The high affinity monoclonal antibody 1F7 disclosed in Duke-Cohan et al. did not inhibit gelatin degradation and invasion by endothelial cells.

21. The Hartel-Schenk et al. reference (*Eur. J. Biochem.* 1991, 196:369) provides no evidence that the disclosed antibodies directed against DPPIV inhibit angiogenesis. Furthermore, since the ability to inhibit angiogenesis is rare among monoclonal antibodies against DPPIV, there is no reasonable expectation that the monoclonal antibodies disclosed in Hartel-Schenk et al. would inhibit angiogenesis.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Date: July 30, 2002

Wen-Tien Chen, Ph.D.

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Wen-Tein Chen, Ph.D.

Professor
Department of Medicine/Division of Neoplastic Diseases
State University of New York
Stony Brook, New York

EDUCATION:

Bachelors of Science degree in 1968 from Tunghai University in Taiwan in field of biology.

Masters of Science degree in 1974 from Northeastern University in Massachusetts in field of embryology.

Doctorate of Philosophy degree in 1979 from Yale University in Connecticut in field of genetics, cellular and developmental biology.

POSITIONS AND HONORS:

1974-1979	Research Assistant, Biology Department, Yale University. (with Dr. J.P. Trinkaus).
1979-1982	Postdoctoral Fellow in the area of membrane chemistry, University of California at San Diego. (with Dr. S.J. Singer).
1982-1984	Director, Cytochemistry Division, Howard University Cancer Center; Assistant Professor, Department of Oncology, Howard University College of Medicine.
1984-1991	Associate Professor, Department of Cell Biology, Georgetown University School of Medicine.
1988-1992	Director, The Cytochemistry & Morphology Core, Lombardi Cancer Center. Georgetown University School of Medicine.
1988-8/1998	Director, Program of Cellular Control of Invasion and Metastasis, Lombardi Cancer Center, Georgetown University School of Medicine.
1992-1993	Sabbatical in Section of Molecular Biology, NIDR, NIH (Dr. Yoshi Yamada).
1992-1998	Professor, Department of Cell Biology, Georgetown University School of Medicine.
8/1998-	Professor, Department of Medicine/Division of Neoplastic Diseases, SUNY @ Stony Brook.
8/1998-	Director, Program of Angiogenesis and Metastasis, Long Island Cancer Institute, SUNY @ Stony Brook.

REVIEW PANELS:

(1). Committee Member:

National Institutes of Health, Cell Biology Study Section (CBY-1), 1989-1993; National Institutes of Health, Biomedical Sciences Study Section (BI3), 1984-1987.

(2). Ad Hoc Committee Member:

Cancer Center Support Grants, Site Visits in 2/12-2/14/02; 10/11–10/13/00; 5/31-6/2/2000; 6/29–7/1/98, 10/6-10/8/97;
NCI Cancer Preclinical Program Project Review Committee, Site Visits in 2/28-3/2/99, 9/16-9/18/96, 1/23-1/25/96;
NCI Special Review Committee, Outstanding Investigator Grants: 12/9-12/11/92;
NIH Special Review Committee: 1/31-2/2/99, 3/1991, 3/1990, 3/1987;
RFA DE-98-008 Genetic Mechanisms in Oral Cancer, 1/31–2/2/1999;
RFA 86-HL-30-L Fibroblast Heterogeneity in Pulmonary Fibrosis, 7/8-7/9/1987;
NIH Pathology B Study Section, Special Study Section Review in 4/1984.

SELECTED PUBLICATIONS:

1. Chen, W.-T. 1979. Induction of spreading during fibroblast movement. J. Cell Biol. 81:684-691.
2. Chen, W.-T. 1981. Mechanism of retraction of the trailing edge during fibroblast movement. J. Cell Biol. 90:187-200.
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4. Chen, W.-T., and S.J. Singer. 1980. Fibronectin is not present in the focal adhesions formed between normal cultured fibroblasts and their substrata. Proc. Natl. Acad. Sci. U.S.A. 77:7318-7322.
5. Chen, W.-T., and S.J. Singer. 1982. Immunoelectron microscopic studies on the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. J. Cell Biol. 95:205-222.
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9. Chen, W.-T., J.-M. Chen. and S.C. Mueller. 1986. Coupled expression and colocalization of 140K cell adhesion molecules, fibronectin, and laminin during morphogenesis and cytodifferentiation of chick lung cells. J. Cell Biol. 103: 1073-1090.
10. Chen, J.-M., and W.-T. Chen. 1987. Fibronectin-degrading proteases from the membranes of transformed cells. Cell 48: 193-203.
11. Chen, W.-T. 1989. Proteolytic activity of specialized surface protrusions formed at rosette contact sites of transformed cells. J Exptl. Zool. 251:167-185.

12. Mueller, S.C., T. Kelly, M. Dai, H. Dai, and W.-T. Chen. 1989. Dynamic cytoskeleton-integrin associations induced by cell binding to immobilized fibronectin. J. Cell Biol. 109:3455-3464.
13. Aoyama, A., and W.-T. Chen. 1990. A 170-kDa membrane-bound protease is associated with the expression of invasiveness by human malignant melanoma cells. Proc. Natl. Acad. Sci. U.S.A. 87:8296-8300.
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15. Chen, W.-T. 1992. Membrane proteases: roles in tissue remodeling and tumor invasion. Current Opinion in Cell Biology. 4:802-809.
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17. Kelly, T., S.C. Mueller, Y. Yeh, and W.-T. Chen. 1994. Invadopodia promote proteolysis of a wide variety of extracellular matrix proteins. J. Cell Physiol. 158:299-308.
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19. Chen, W.-T., C.-C. Lee, L. Goldstein, S. Bernier, C. H.L. Liu, C.-Y. Lin, Y. Yeh, W. L. Monsky, T. Kelly, M. Dai, J.-Y. Zhou, and S. C. Mueller. 1994. Membrane proteases as potential diagnostic and therapeutic targets for breast malignancy. Breast Cancer Research and Treatment. 31:217-226.
20. Chen, W.-T. 1996. Proteases associated with invadopodia, and their role in degradation of extracellular matrix. Enzyme Protein 49: 59-71.
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28. Chen, W.-T., and J.-Y. Wang. 1999. Specialized surface protrusions of invasive cells, invadopodia and lamellipodia, have differential MT1-MMP, MMP-2 and TIMP-2 localization. In Conference on Inhibition of Matrix Metalloproteinases: Therapeutic Applications. Annals New York Academy of Science 878: 361-371.
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Table 1. Effects of monoclonal antibodies against DPPIV on gelatin-degradation and cellular invasiveness.

mAb	antibody type	epitope*	Inhibition of gelatin-degradation**	Inhibition of invasion***
E1	rat IgG2a	DPPIV	-	-
E3	rat IgG2a	DPPIV	-	-
E5	rat IgG1	DPPIV	-	-
E6	rat Ig ?	DPPIV	-	-
E7	rat IgG2a	DPPIV	-	-
E10	rat Ig ?	DPPIV	-	-
E19	rat IgG2a	DPPIV	+	+
E20	IgM	DPPIV	-	-
E26	rat IgG2a	DPPIV	+	+
E30	rat IgG2a	DPPIV	-	-
E31	rat Ig ?	DPPIV	-	-
E33	rat IgG2a	DPPIV	-	-
E96	rat Ig ?	DPPIV	-	-
E97	rat Ig ?	DPPIV	-	-
E116	rat Ig ?	DPPIV	-	-
F2	rat IgG2b	DPPIV	-	-
F4	rat IgG2a	DPPIV	-	-
F8	rat IgG2b	DPPIV	-	-
F9	rat IgG2b	DPPIV	-	-
F10	rat IgG2b	DPPIV	-	-
F12	rat IgG2a	DPPIV	-	-
F20	rat IgG2a	DPPIV	-	-
F24	rat IgG2b	DPPIV	-	-
F87	rat Ig ?	DPPIV	-	-
F95	rat Ig ?	DPPIV	-	-
F102	rat Ig ?	DPPIV	-	-
1F7	mouse IgG2b	CD26	-	-
202.36 (Ancell)	mouse IgG2b	CD26	-	-
M-A261 (BD PharMingen)	mouse IgG1	CD26	-	-

* Epitope was determined by Western immunoblotting.

** Gelatin-degradation was determined by antibody inhibition of the release of fluorescently labeled denatured type I collagen (gelatin) by immunocaptured DPPIV-seprase complexes,

*** Invasion was determined by the inhibition of endothelial cell invasion into type I collagen gel that overlaid on the monolayer wound model as described in Figure 2h-j of the specification as originally filed.

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Proteolytic-substrate soluble assay

The fluorescein labeled gelatin (DQ-gelatin, Molecular Probes) was used as the substrate for the DPPIV-seprase complex in immunoprecipitates. The gelatin was heavily labeled with DQ-fluorescein in which fluorescence was quenched; increase in fluorescent signal indicates cleavage and release of gelatin peptides into solution. Thus, increase in fluorescence was proportional to proteolytic activity. Briefly, 96-well microtiter plates (Costar, Corning, NY) were coated with rabbit anti-rat antibodies (20 μg/ml) and incubated at 37°C for 2 h. Wells were then rinsed with wash buffer (PBS, 0.1% Tween 20), and excess binding sites were blocked with blocking buffer (2% bovine serum albumin in PBS) for 1 h at 37°C. Blocking buffer was removed; wells were coated with anti-human DPPIV mAbs (E3; 10 μg/ml), anti-human seprase mAbs (D8 or D28; 10 μg/ml), or control antibody in PBS and incubated at 37°C for 2 h. Membrane extracts or WGA (wheat germ agglutinant)-enriched protease supernatants derived from HUVEC (human umbilical vein endothelial cells) or MDA-MB-436 human breast carcinoma cells were added and incubated for 1 h at 4°C. The unbound material was removed; wells were washed three times with wash buffer. Gelatin-degrading activity of the immuno-captured DPPIV-seprase complexes were assayed using 100 μl of DQ-gelatin (25 μg/ml in PBS, pH 7.4) with or without small inhibitors or mAbs specific for DPPIV (0.1-10 μg/ml). The change of fluorescent intensity was read at wavelength of 485 (excitation)/538 (emission) using a Microplate Spectrophotometer System (Molecular Devices).